RESISTANCE AND SENSITIVITY PATTERN OF STAPHYLOCOCCUS AUREUS; A STUDY IN LADY READING HOSPITAL PESHAWAR

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ABSTRACT

Objective: *This study was done to determine the sensitivity and resistance pattern to the most common antibiotics in use.*

Methodology: *The data for this retrospective study was obtained and analysed from October 2010 to October 2011 in Lady Reading Hospital, Peshawar. Purposive sampling and univariate analysis was done. 2058 samples were cultured for sensitivity using Kirby Bauer's Diffusion technique and in a period of one year, growth of Staphylococcus aureus was obtained in 723 samples. These 723 growths were obtained from clinical isolates of pus (699), blood (16) and urine (8). Fourteen drugs from five different classes of antibiotics were tested for sensitivity against Staphylococcus aureus.*

Results: *Most of the growth was obtained from pus (96.7%). Resistance was high to all groups of antibiotics except glycopeptides. There was no case of Vancomycin Resistant Staphylococcus aureus (VRSA). Out of 723 samples, 228 (31.5%) were Methicillin Resistant Staphylococcus aureus (MRSA). The resistance to a representative antibiotic of each group is as follows: Ciprofloxacin (Fluoroquinolone) 51.7%, Cephradine (Cephalosporin) 46.2%, Amoxicillin+Clavulanic Acid (Penicillin group) 45.6%, Imipenem+Cilastatin Sodium (Carbapenem) 42.0% and Teicoplanin (Glycopeptide) 19.8%.*

Conclusion: *Resistance of Staphylococcus aureus to Teicoplanin and most of the antibiotics is high and strict guidelines are required to control unnecessary prescriptions and over the counter sale of antibiotics.*

Key Words: *Antibiotic resistance, Staphylococcus aureus, Resistance, Sensitivity*

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INTRODUCTION

Antibiotic resistance is growing worldwide and its impact is more in poor countries where infections are high due to poverty, unhygienic environment and poor health facilities. In such settings, the morbidity and mortality rate is also higher due to infectious diseases. The challenge of resistance also persists in developed countries. There is also emergence of multidrug resistant organisms which have made the situation worse[1]. *Staphylococcus Aureus*, a facultative anaerobic gram positive coccus, is a part of skin flora and causes skin infections such as cellulitis, scalded skin syndrome as well as life threatening conditions like meningitis, Pneumonia, Endo-carditis and Sepsis. It is best known to be the cause of nosocomial infections. Antibiotics are life saving drugs and it must be remembered that not only the organism but the excessive unnecessary use of these life saving drugs is also a major cause

of resistance. *Staphylococcus aureus* has developed multiple ways to become resistant to many antibiotics. This involves production of beta lactamase enzymes, changes in cell wall structure and genetic mutations[2,3]. The organism also has one of the most dangerous strains known as Methicillin Resistant *Staphylococcus aureus* (MRSA) which are resistant to most of the antibiotics except Glycopeptide group e.g. Vancomycin and Teicoplanin. But resistance has been reported against Vancomycin[4,5] and Teicoplanin[6] as well, therefore there is a need for a surveillance program in every country to assess the level of resistance. In Europe, Antimicrobial Resistance Surveillance System (EARSS) is a surveillance network which works in 31 countries and consists of over 400 laboratories. It reports antimicrobials resistance patterns every 18 months from Europe[7]. In Pakistan, antibiotics are sold over the counter without any prescription[8], moreover culture and sensitivity is also not routinely done and physicians and chemist are using the drugs without thinking about long term consequences like resistance. There is no surveillance network to check the prescriptions and to compile reports from laboratories to determine the antibiotic resistance patterns of different organisms. Health education and schools of hygiene are very limited and so is the number of studies done till now to report the antibiotic resistance in the Khyber Pakhtunkhwa Province. Therefore, we selected a major tertiary care hospital of Peshawar for this purpose to report for the first time the pattern of resistance and sensitivity found here. The results of this study will help us to know the current situation and would also give us an opportunity to attract researchers to find the causes of increased resistance.

METHODOLOGY

This was a retrospective study over a period of one year i-e from October 2010 to October 2011. During this period, a total of 2058 clinical isolates were received for culture and sensitivity at the Pathology Laboratory of Lady Reading Hospital Peshawar. The samples were collected from pus, blood, urine, wound sites and other body fluids using sterile techniques. Purposive sampling was done. All samples received for culture and sensitivity were included in the study. Samples which did not yield *Staphylococcus aureus* growth were excluded altogether. Out of 2058 samples, growth of *Staphylococcus aureus* was obtained in 723 samples. Of these 723 samples, 699 were from pus, 16 from blood and 8 from urine. Incubation of the samples was done on Mannitol Salt agar, McConkey's agar and Sheep blood agar at 37°C for 24 hours. CLED agar plates were used for culture of urine samples. Colony morphology, Gram staining using crystal violet dye, catalase test and coagulase test was used to identify *Staphylococcus aureus*. Following revised 2010 Clinical and Laboratory Standard Institute guidelines, antibiotic susceptibility testing using Kirby Bauer's disc diffusion technique and interpretation of inhibition zone diameters (in mm) was done. The growth was subjected to a total of 14 drugs from five different classes of antibiotics to check the sensitivity pattern of the *Staphylococcus aureus*. Data entry and analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done and percentages for the two aspects of the variable i-e sensitivity and resistance, were calculated for each antibiotic. The drug discs used were as follows: Penicillin Group: Methicillin (Oxacillin 5µg), Amoxicillin (20µg), Amoxicillin + Clavulanic acid (20/10µg), Piperacillin + Tazobactam (100/10µg), Glycopeptides: Vancomycin (30µg), Teicoplanin (30µg), Carbapenems: Meropenem (10µg), Imipenem + Cilastatin Sodium (10µg), Cephalosporins: Cephradine (25µg), Ceftriaxone (30µg), Ceftazidime (30 µg), Cefoperazone (75µg) and Fluoroquinolones: Ciprofloxacin (5µg) and Enoxacin (10µg).

RESULTS

The results of the study are given in Table 1 and 2.

Table 1 shows the number and type of those isolates in which the growth of *Staphylococcus aureus* was obtained. Resistance and sensitivity of 14 antibiotics have been shown in Table 2. Only generic names of antibiotics have been used and the class to which the drug belongs,

Table 1: Type of clinical isolates for culture and sensitivity

Type of Clinical Isolate	n	%
Pus	699	96.70
Blood	16	2.21
Urine	8	1.09
Total	723	100.0

Table 2: antibiotic drug resistance and sensitivity in *Staphylococcus aureus* isolates

S No.	Class of Antibiotic	Generic name of Antibiotic	Resistant		Sensitive		Drug tested on
			n	%	n	%	(number of samples)
1.	Penicillin Group	Methicillin	228	31.5	495	68.5	723
2.	Penicillin Group	Amoxicillin	713	98.6	10	1.4	723
3.	Penicillin Group	Amoxicillin + Clavulanic Acid	330	45.6	393	54.4	723
4.	Penicillin Group	Piperacillin + Tazobactam	57	34.6	108	65.4	165
5.	Glycopeptide	Vancomycin	0	0.0	723	100	723
6.	Glycopeptide	Teicoplanin	143	19.8	580	80.2	723
7.	Carbapenem	Meropenem	296	40.9	427	59.1	723
8.	Carbapenem	Imipenem + Cilastatin Sodium	304	42.0	419	58.0	723
9.	Cephalosporin	Cephradine	334	46.2	389	53.8	723
10.	Cephalosporin	Ceftriaxone	365	50.5	358	49.5	723
11.	Cephalosporin	Ceftazidime	148	98.0	3	2.0	151
12.	Cephalosporin	Cefoperazone	143	94.7	8	5.3	151
13.	Fluoroquinolone	Ciprofloxacin	374	51.7	349	48.3	723
14.	Fluoroquinolone	Enoxacin	111	73.5	40	26.5	151

has been mentioned.

DISCUSSION

We found 228 (31.5%) cases of MRSA (Methicillin Resistant *Staphylococcus aureus)* during the 12 months study. Our results differ from studies conducted elsewhere in Pakistan because the incidence varies a lot among hospitals but incidence is mostly reported higher in big cities[9,10,18]. In a multicentre study conducted on 792 clinical isolates of *Staphylococcus aureus* from 8 laboratories all over Pakistan i.e. Karachi, Peshawar, Lahore, Sukkhur, Islamabad, Quetta, and Mirpur, Azad Kashmir, the incidence of MRSA was found to be 2-61% while specifically in Peshawar it was 36%. In another study conducted in Karachi from Januray 2009 to December 2009, there were 174 (38.6%) MRSA cases out of a total of 450 isolates. It is worth mentioning that it also reported one case of VRSA (Vancomycin Resistant *Staphylococcus aureus*)[11]. In another study conducted from December 2009 to January 2008 in CMH Pano Akil, only 19.8% cases were that of MRSA. We did not find any strain of *Staphylococcus aureus* resistant to Vancomycin making it the only antibiotic to which all strains were susceptible and this has been found out in other studies also[11,13,14,16]. We did encounter higher i-e. 19.8% resistance to Teicoplanin and further research, in isolating such resistant strains of *Staphylococcus aureas* as well as investigating the mechanism of resistance of these strains to Teicoplanin, should certainly be done. Teicoplanin has otherwise very good activity against *Staphylococcus aureus* as seen in other studies in Pakistan[15,16]. In a study conducted in Islamabad during the period from December

2007 to August 2008, only 3% resistance against Teicoplanin was found[17]. Tertiary Hospitals should take more steps to ensure hygiene and decrease the burden of these deadly strains.

Our study shows that *Staphylococcus aureus* has less sensitivity to Penicillin group of antibiotics[16]. We found that only 1.4% organisms were susceptible to Amoxicillin. With the addition of Clavulanic acid to Amoxicillin, the sensitivity increased from 1.4% to 54.4%. Therefore, it suggests that a great number of strains produce Beta lactamases enzymes and combination of Amoxicillin with Clavulanic acid is much better. The combination of Piperacillin with Tazobactam was also a better combination (65.4% sensitivity) and it was found more effective than Amoxicillin + Clavulanic Acid. It must be noted that in many studies like this, resistance to Beta-lactam Antibiotics was found higher than other groups. In a multicentre study in Pakistan, during the period from April 2006 to March 2008, resistance to Oxacillin, Penicillin and Ampicillin was found to be 100% while that of Cephalothin was 92.4%[18].

Meropenem and Imipenem+Cilastatin Sodium did not differ much in their activity against *Staphylococcus aureus* with 59.1% and 58.0% sensitivity respectively. These antibiotics are resistant to degradation by beta lactamases and that is why their sensitivity is more than Amoxicillin + Clavulanic Acid. Imipenem + Cilastatin Sodium, has been shown in studies of Japan[19] and USA[20], as very active against *Staphylococcus aureus.*

Cephradine – a first generation Cephalosporin has very good activity against gram positive organisms. We found 46.2% resistance to Cephradine making it superior than the third generation Cephalosporins and Fluoroquinolones used in our study but inferior to Carbapenems. Resistance against Cefoperazone and Ceftazidime has come very high in our study[21]. Studies have shown that if Cephalosporins are combined with Beta lactamases inhibitors, their efficacy can be greatly increased[22,23].

Although Fluoroquinolones are not considered the first line drugs for gram positive organisms yet they have a broad spectrum and Ciprofloxacin, the earlier and the most common drug used of this group in our hospitals, had excellent activity against *Staphylococcus aureus* including Methicillin resistant strains[24] when it was introduced but Topoisomerase and other genetic mutations have made *Staphylococcus aureus* resistant to these old drugs – Ciprofloxacin and Enoxacin[4,25]. We encountered 51.7% resistance to Ciprofloxacin. A study from Karachi reported 63.7% resistance to Ciprofloxacin in 2009.[9] The resistance to Enoxacin was very high 73.5%, but it is not unusual as Enoxacin is less effective than Ciprofloxacin. Studies have reported that the fourth generation Fluoroquinolones like Moxifloxacin have little resistance but it is not widely used here and therefore not tested[4,16].

CONCLUSION

Incidence of MRSA was 31.5% which is very high. We conclude that except 3 antibiotics i-e. Vancomycin, Teicoplanin and Methicillin, the resistance to all the other antibiotics was more than 40%. Moreover, the resistance to Amoxicillin, Cefoperazone and Ceftazidime is more than 90% and should not be prescribed for such infections. Guidelines regarding the use of antibiotics must be made and strict laws also implemented to stop the free use of these drugs by quacks and chemists also. The hospitals should improve their hygiene to avoid spread of these dangerous organisms. National guidelines would ideally be based on several larger comprehensive studies and the authors recommend such studies be done especially in Khyber Pakhtunkhwa involving other hospitals also. Education of doctors is very necessary now as new pattern of resistance and sensitivity require them to know the effective drugs for each organism and do not prescribe unnecessary and ineffective antibiotics. If this is not done, it is very likely that in future these life saving drugs would be useless as a result of increased resistance.

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CONTRIBUTORS

MN Supervised the whole project, revised the article and gave the final approval. MA wrote the article for the first time, did the data analysis, literature review and edited the article. SMN took part in literature review, data analysis and interpretation. SHA, MZUIK & AK took part in study design, data collection, entry and revision of the article in every stage. MUK reviewed the article in every stage and helped a lot in study design, literature review and final revision of the article. The project was self funded. All authors have taken part in its writing, interpretation of data revision and all authors approve its final version.